S2 Result. Optimization of multiplex PCR screen
The tet(A)\textsuperscript{wt} allele cannot be easily distinguished from tet(A)\textsuperscript{ΔtetR} purely on phenotypic observations (see S6 Table). To generate a screening procedure to identify tet(A)\textsuperscript{wt} and tet(A)\textsuperscript{ΔtetR}, we optimized a simple multiplexed PCR screen that both detects presence of tet(A) and identifies the tetR(A) allele. The high similarities present in tetR(A) sequence at and around the 24-bp deleted region did not allow us to design PCR primers that could efficiently amplify only one of the two alleles. Instead, size-discrimination of the PCR product amplifying part of tetR(A) was used to distinguish the different tet(A) alleles. The multiplex PCR was tested on 22 whole-genome sequenced isolates (13 with tet(A) from a collection of UPEC and blood isolates [1] and 9 without tet(A) from previously screened isolates, included in S2 Table) and successfully identified all tet(A)-carrying strains, as well as the correct tet(A) allele (subset shown in S8A Fig). Multiplex tet(A) alleles can be present in an isolate but a single tet(A)\textsuperscript{wt} copy can allow for spontaneous, clinically-relevant TGC resistance to develop. We showed that a tet(A)\textsuperscript{wt} allele present at ≥10% of all copies of tet(A) could be correctly detected in our PCR screen, which also detected the presence of both alleles in one E. coli isolate (S8B and S8C Fig).

References